



## **WATER RESOURCES RESEARCH GRANT PROPOSAL**

**Title:** Denitrification in Wetlands Receiving Mississippi River Freshwater Diversion: Water Quality Aspects

**Focus categories:** WQL, NPP, NC, NU, WL

**Keywords:** Water quality, freshwater diversion, nitrogen, biogeochemistry

**Duration:** March 1, 2000 through February 28, 2001

**Federal funds requested:** \$17,800

**Non-Federal (matching) funds pledged:** \$35,720

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**Congressional district:** Sixth Congressional District

### **Statement of critical regional water problems**

Louisiana coastal waters are experiencing low oxygen conditions, or dead zones. The dead zone is attributed to excessive nutrients from the Mississippi River which cause an increase in plankton activity. As the plankton die, sink to the bottom and decay, oxygen is removed from the water column creating a lifeless or dead zone. Agriculture is thought to be the primary source of nutrients flowing from the Mississippi River into the Gulf of Mexico. Nitrate concentration in river water has tripled over the past several decades. We propose to study denitrification or nitrate removal in wetlands at two Mississippi freshwater diversion sites. Diverting Mississippi waters into coastal drainage basins will lower amount of nitrate entering offshore waters.

### **Statement of the results, benefits, and/or information expected to be gained**

Nutrient loading of the Mississippi River is reported as the major factor in the Gulf hypoxia problem. Efforts are underway to implement agricultural best management practices (BMP) for reducing nitrate input into the Mississippi River watershed. We propose to quantify denitrification rates of nitrate removal in Louisiana coastal wetland receiving freshwater input from Mississippi River freshwater diversions. The significance of denitrification in reducing nitrate levels in water entering coastal drainage basins will be determined. The benefits of coastal restoration projects (freshwater diversion through estuaries) in nitrate removal and as a tool for combating the dead zone will be documented.

## **Nature scope and objective of the research**

Low oxygen or hypoxic water in coastal or marine ecosystems is receiving increasing attention. Globally low oxygen cause more fish deaths or shifts in species diversity than any other single agent in coastal and marine environments. It is reported that there is no environmental variable of more ecological importance to coastal marine ecosystem that has changed so significantly over such a short period of time (Diaz and Rosenberg 1995). The increase in hypoxic in the worlds oceans, bays, and estuaries is attributed to pollutants such as fertilizer and sewage carrying nutrients which nourish phytoplankton.

Along the Louisiana Gulf Coast recent attention has been given to problems associated with nutrient loading to coastal water bodies. Gulf Coast scientists have documented a large area of low oxygen water sitting along the bottom of the Gulf of Mexico along the Louisiana Coast (Turner and Rabalais 1994). This low oxygen (dead zone) has implications to Louisiana seafood industry. The dead zone has been attributed to the excessive nutrients in the Mississippi River which ultimately enters the Gulf. Scientist from the Louisiana Universities Marine Consortium reported that pollutants from the Mississippi River have produced the three largest dead zones on record since mapping of oxygen levels began 11 years ago. Difficulty with respiration is a major problem animals face in hypoxic water. Organisms either flee such areas or die from lack of oxygen (Alder 1996). The dead zone in the Gulf of Mexico runs from a depth of approximately 10 to 30 meters covering a large area extending from the mouth of the Mississippi River. It is estimated that the dead zone covers an area of some 7,000 square miles.

In nitrogen limited oceanic food webs increased phytoplankton production is reported to be the likely result of increased nitrogen loading (Harris 1986). The productivity of coastal plankton in the Gulf Coast region is also reported to be limited by nitrogen (Turner and Rabalais 1994). Accordingly, scientists have attributed excessive nutrients from the Mississippi River to the increase in plankton activity in the Gulf of Mexico in the warmer months. Plankton use oxygen from the water while growing and remove more as they die and sink to the bottom. The dead zone generally forms in May reaching a peak in midsummer and declining during the fall.

Several recent conferences attended by federal and state regulatory officials have been held to gather information on the dead zone and identity potential sources of nutrient enrichment to the region. The source of nutrients in the Mississippi River which discharges into the Gulf is reported to be the Corn Belt region of the Upper Mississippi Valley. Nitrogen is currently applied to agricultural lands in the Mississippi drainage basin at a rate four times that which was applied in 1960. The amount of nitrate entering the Gulf from the Mississippi River has tripled during that period (Turner and Rabalais 1991). Approximately 45 percent of the nitrate that reaches the mouth of the Mississippi River appear to be entering from the upper portion of the river. Only 19 percent of the nitrate is from the Lower River Valley. The U.S. Geological Survey reports that once the nitrate enters the river, it apparently remains in the river valley. Nitrate-N in the Mississippi River at New Orleans on the order of 100  $\mu\text{g at/l}$ .

Nitrogen entering coastal wetland systems can undergo a series of transformations. Mudflats and marsh sediment within estuarine ecosystems have the potential to remove significant nitrate from the water column directly through denitrification (Koch et al. 1992; Seitzinger and Nixon 1985). Nitrification-denitrification reactions in the Mississippi deltaic plain and in offshore sediment potentially can remove appreciable amounts of nitrogen thus improving surface water quality. Inorganic ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) entering these wetlands can be (1) nitrified ( $\text{NO}_3^-\text{-N}$ ), (2) assimilated, or (3) absorbed by bottom sediment. Nitrification can occur in the water column, in the sediment surface oxidized layer and around the marsh plant rhizosphere (Jenkins and Kemp 1984; Reddy and Patrick 1984). The nitrate ( $\text{NO}_3^-\text{-N}$ ) produced can diffuse to a reduced soil layer and be denitrified and released to the atmosphere as dinitrogen gas ( $\text{N}_2$ ) or nitrous oxide gas ( $\text{N}_2\text{O}$ ).

A wide range in denitrification rates is reported for aquatic sediments (Seitzinger 1990). Lowest reported denitrification rates are in deep sea sediments ( $.03$  to  $2.4 \mu\text{mol N m}^{-2} \text{h}^{-1}$ ). Rates for continental shelf sediments are greater up to  $20 \mu\text{mol N m}^{-2} \text{h}^{-1}$ . Rates in estuaries range from  $5$  to  $250 \mu\text{mol N m}^{-2} \text{h}^{-1}$ . Some of the highest reported denitrification rates are polluted estuarine sediments ( $>500 \mu\text{mol N m}^{-2} \text{h}^{-1}$ ). Reported denitrification in eutrophic streams and rivers range from  $40$  to  $212 \mu\text{mol N m}^{-2} \text{h}^{-1}$ .

In the past, denitrification losses determined nitrogen balance studies, have been estimated by indirect laboratory and field techniques. By far, the most popular method for measuring denitrification has been the acetylene blockage technique because of its sensitivity and rapid gas chromatographic procedures (Duxbury 1986). In the past few years, major limitations to the  $\text{C}_2\text{H}_2$  method have been reported. Researchers have shown that  $\text{C}_2\text{H}_2$  inhibits nitrification, affects soil organisms, decomposes in soil and is not uniformly dispersed throughout the saturated soil (Keeney 1986; Rolston 1986). The use of highly labelled N compounds permits the direct measurement of nitrogen gases produced during denitrification. Mass spectrometry is used to quantify  $\text{N}_2$  and  $\text{N}_2\text{O}$  evolved or gas chromatography can measure directly the  $\text{N}_2\text{O}$  flux from such wetland systems (Siegel et al. 1982).

Nitrogen input-output budgets are an important approach to evaluation of ecosystem processes. Nitrogen is generally considered to be the nutrient that limits productivity in coastal waters and therefore, input-output analysis of N is critical to evaluation of ecosystem productivity and its response to natural and nonpoint source input. The recent recognition of high rates of anthropogenically-N input into Gulf Coast waters has raised concern. Wetlands that are N saturated do not absorb N and therefore transmit more N to aquatic ecosystems. Moreover, excess N can induce a variety of deleterious chemical and biological changes to ecosystems.

Currently there is little information on the fate of the elevated nitrogen entering Gulf Coast waters through Mississippi River discharge. Quantification of denitrification rates would provide critical information for evaluating the true impact of nitrate on these coastal ecosystems. We hypothesize that denitrification removes significant amounts of

nitrate from Mississippi River water entering Louisiana Coastal Zone, and that marsh restoration projects (freshwater diversion) will enhance nitrate removal.

## **Objectives**

We propose to determine denitrification or nitrate removal in two basins receiving Mississippi freshwater diversion.

Specifically we will determine the following:

- 1) Quantify denitrification rates or nitrate processing capacity.
- 2) Quantify emission of dinitrogen and nitrous oxide to the atmosphere resulting from denitrification.
- 3) Identify benefit of diverting Mississippi River waters through coastal marshes in nitrate removal and as a tool in combating the dead zone.

## **Methods, procedures, and facilities**

We propose to quantify denitrification rates in wetland at the Caernarvon diversion and at Davis Pond. The Caernarvon freshwater diversion structure (the largest of six diversion currently in operation) is located on the east bank of the Mississippi River below New Orleans near Caernarvon, La., at river mile 81.5. The control structure is a five box culvert 4.6 meters wide with vertical lift gates. The structure currently introduces up to 4,000 cubic ft per sec of water. Actual freshwater discharge which is recorded varies depending on time of year. The Caernarvon diversion delivers water into Breton Sound estuary, which consists of 1,100 km<sup>2</sup> of fresh, brackish, and saline wetlands. The estuary is hydrologically bounded to the west by the Mississippi River levee, to the north by natural levees of Bayou La Loutre, and to the east by spoil banks of the Mississippi River Gulf Outlet. The estuary has open connection to the Gulf of Mexico.

The Davis Pond project is scheduled to be operational in 2000. The structure will introduce 10,000 cubic ft per sec of freshwater into Northern Barataria Basin. The water will flow through Lake Cataouche and Lake Salvador. We propose to monitor change in nitrate concentration as water moves through wetlands at the two freshwater diversion sites. Additional diversion projects are being planned to reduce the rapid rate of marsh deterioration in the Mississippi River Delta (Figure 1). An added benefit to such Mississippi River freshwater diversion could be a reduction in amount of nutrients entering offshore areas.

Bottom sediment samples and/or cores will be taken along transects from each of the regions described above for determining denitrification rates. The methods which will be used are described in the following section. The sites will include Big Mar and Lake Lery at Caernarvon, and Lake Cataouche and Lake Salvador of Davis Pond (the primary water bodies being impacted by the diversion projects).

## **N<sub>2</sub> and N<sub>2</sub>O Evolution (Acetylene Inhibition)**

Seasonally beginning in the spring of 2000 denitrification rates in marsh soils and sediment will be determined using the acetylene inhibition technique (Sorensen 1978; Knowles 1979). We recognize that this procedure has limitations, but its simplicity will allow for the screening of a large number of sites. This method measures the reduction of NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> by blocking the reduction of N<sub>2</sub>O to N<sub>2</sub> with acetylene (C<sub>2</sub>H<sub>2</sub>) and determining N<sub>2</sub>O in the headspace. We will compare or calibrate against direct measurements of denitrification losses determined using added N-15 labelled nitrate (Lindau et al. 1988). Using the acetylene inhibition technique, the evolution of N<sub>2</sub>O and N<sub>2</sub> will be determined in a series of cores collected from each of the environments. The techniques will be coupled with techniques previously developed for the measurement of N<sub>2</sub>O flux from Louisiana wetlands (Smith et al. 1981, 1982).

Seasonally (6 times per year) ten cores to a depth of 10 cm (diameter 6.0 cm) will be collected from two locations in each study area. A total of 40 cores (10 at each of the two locations within a study area) will be collected per study area per sampling date.

Two different treatments will be applied to (6) six replicates each from the twelve cores taken from each sampling site. Treatment 1 cores will be maintained in a vertical position in incubation chambers and amended with water taken from the site at which the cores were taken using the incubation procedure outlined by Koch et al. 1992. In Treatment 2 the water will be amended or enriched with 300 µg at/l NO<sub>3</sub>-N (3 times the level in river water). Applying added NO<sub>3</sub><sup>-</sup> will allow for the determination of potential denitrification rates in the sediment when there is an elevated source of nitrate in the overlying water column. N<sub>2</sub>O will be determined using an electron capture gas chromatograph (Lindau and DeLaune 1991).

## **Gaseous Denitrification Losses (Direct Measurement)**

Sediment cores (6) will be taken seasonally from three sites within each study area for measuring gaseous denitrification losses. N-15 labelled KNO<sub>3</sub> (50 atom %) will be dissolved and added to the overlying water at several rates to simulate N concentrations found in river water.

Emissions of <sup>15</sup>N<sub>2</sub> and N<sub>2</sub>O will be measured at 2-3 day intervals after N additions. Measurements will continue until emissions of <sup>15</sup>N<sub>2</sub> and N<sub>2</sub>O are below instrument/method detection limits. At each sampling date, cores will be placed in a gas diffusion cylinder (gasketed PVC end caps) and zero-time headspace gas samples will be collected immediately after cap placement. Treatment and control cylinders will be sealed and <sup>15</sup>N<sub>2</sub> and N<sub>2</sub>O flux measured over time. Sediment oxygen uptake will also be measured at the time of gaseous collection. Headspace gas samples will be removed through a rubber septum sealed in the end caps with a gas-tight syringe and hypodermic needle. Collected gas samples will be transferred into evacuated glass Vacutainers (100 mm length by 16 mm I.D.) and the septum needle puncture sealed with silicon rubber. To

prevent atmospheric contamination a slight overpressure of sample gas will be injected into each Vacutainer.

Labelled  $N_2$  and  $N_2O$  gas concentrations will be determined by mass spectrometry and gas chromatography. Nitrous oxide concentrations will be measured on a Perkin-Elmer 8410 gas chromatograph (GC) fitted with an electron capture detector. A Chromosorb 106 (80/100 mesh) column (0.006 x 1.8 m) will be used and the detector temperature will be  $395^{\circ}C$  (Lindau et al. 1988). Nitrous oxide fluxes (F) from the soil surface to the atmosphere will be estimated using the following closed-chamber equation (Rolston 1986):

$$F = (V/A)(273/T)(\Delta C/\Delta T),$$

where V is the core headspace volume, A is the core soil-surface area,  $\Delta C/\Delta T$  is the change in  $N_2O$  concentration per unit of time and T is the absolute temperature of the headspace gases.

Stable N isotopic distribution (28, 29 and 30 masses) of the collected  $^{15}N_2$  (controls and treatments) will be determined on a Finnigan Mat Delta E gas isotope ratio mass spectrometer fitted with a dual inlet and a triple collector. The inlet system will permit direct injection of a sample gas ( $2\text{ cm}^3$ ) into an evacuated manifold. A  $LN_2$  trap will remove  $CO_2$ ,  $H_2O$  and  $N_2O$  contaminants and a hot Cu furnace ( $230^{\circ}C$ ) will remove  $O_2$  from the collected headspace gas samples (Siegel et al. 1982). The emissions of  $N_2$  from the sediment to the atmosphere will be calculated using the equations described by Mulvaney and Boast (1986) for mass spectrometers equipped with triple collectors. The percent of the total  $N_2$  evolved due to  $NO_3^-$ -N addition will be calculated using the  $NO_3^-$ -N atom %  $^{15}N$  enrichment and the  $^{15}X_N$  value (mole fraction of  $^{15}N$  in  $NO_3^-$  pool) calculated from the Mulvaney and Boast (1986) equations. Entrapped  $^{15}N_2$  and  $N_2O$  will also be determined using the method of Lindau et al. (1991). Accumulation of sediment-entrapped  $^{15}N_2$  and  $N_2O$  will be estimated at 5 day intervals after  $NO_3^-$ -N application to the water column. At each sampling date, two additional water/sediment cores for each nitrogen treatment will be sealed and after the zero-time gas samples are collected the cores will be vigorously shaken on a mechanical shaker to release sediment-entrapped  $^{15}N_2$  and  $N_2O$ . After shaking, the headspace gases will be sampled a second time which will allow an estimation of the concentrations of entrapped gases to be made. At the end of the sampling period, sediment cores will be thoroughly mixed and total N and N-15 content will be determined. Total nitrogen and N-15 content will be determined by acid digestion, steam distillation and mass spectrometer procedures (Mulvaney 1993). An N-15 input-output balance will be calculated from the N-15 measurements. Total N-15 added to the cores (labelled  $NO_3^-$  additions) will be compared to N-15 output components  $^{15}N_2 + ^{15}N_2O$  evolved plus  $^{15}N_2 + ^{15}N_2O$  entrapped plus total Kjeldahl N-15 in water and sediment column.

## Nitrification-Denitrification in Sediment Water Column (Isotope Dilution)

Replicate (6) sediment cores (15 cm diameter) will be collected during summer and winter months from the marsh soil and bottom sediment at selected wetland locations receiving freshwater discharge. In the laboratory, the sediment cores including water columns will be subjected to nitrogen addition,  $^{15}\text{N}$ -labelled  $\text{NO}_3^-$  (40 atom %), at several rates will be added to the sediment water column (DeLaune and Smith 1987). Samples of the floodwater will be collected over time and filtered through 0.45  $\mu\text{m}$  filters, and the filtrates stored at  $0^\circ\text{C}$  prior to analysis. Concentrations of the dissolved inorganic  $\text{NO}_2^- + \text{NO}_3^-$  will be determined by reduction to  $\text{NH}_4^+$ , with Devarda's alloy and distillation into boric acid. The distillates will be acidified, concentrated to 1 mL, and stored in closed vials prior to N isotope ratio analysis.

The rate of production (nitrification) or reduction of  $\text{NO}_3^-$  will be determined from the changes with time in the concentration and the isotopic ratio for the  $\text{NO}_2^- + \text{NO}_3^-$  in the overlying water. If only nitrification and  $\text{NO}_3^-$  reduction are responsible for changes in the  $\text{NO}_3^-$  pool in the sediment-water columns, their rates can be calculated using the equations outlined by Koike and Hattori (1978) and DeLaune and Smith (1987). Briefly the equations are:

$$N_2 - N_1 = Z - Y \quad [1]$$

$$N_2X_2 - N_1X_1 = ZX_a - YX \quad [2]$$

where  $Y = \text{NO}_3^-$  reduction;  $Z =$  production of  $\text{NO}_3^-$  by nitrification;  $N$  and  $X =$  the concentrations of  $\text{NO}_3^-$  and its  $^{15}\text{N}$  content, respectively, and  $X$  and  $X_a$  are the average isotopic content of the  $\text{NO}_3^-$  and  $\text{NH}_4^+$  between observations  $t_1$  and  $t_2$ , respectively. All data will be subjected to analysis of variance and Duncan's multiple range test ( $P = 0.05$ ) using the Statistical Analysis System (SAS, 1988).

## Relationship of Denitrification to Sediment Organic Content and Labile Carbon

Total sediment respiration during an aerobic incubation will be used to estimate sediment labile C at each of the sites sampled. Incubating sand with moist sediment (20:1 dry mass ratio) will result in aerobic conditions and C mineralization to  $\text{CO}_2$ , which has been shown to be closely correlated with the amount of C mineralized under anaerobic conditions (Crozier et al. 1995). Incubation microcosms for determining estimates of labile carbon will consist of sealed 140-ml glass jars with a 1.00 M KOH alkali trap. After 7 d at  $23^\circ\text{C}$  in the dark, alkali traps will be removed and  $\text{BaCl}_2$  added to precipitate carbonates. Samples will be titrated with 0.25 M HCl using a phenolphthalein indicator (Anderson 1982).

Total sediment carbon content will be determined by dry combustion following acid treatment to remove any carbonates.

Measured denitrification rates will be correlated to labile and total organic carbon content of the sediment at the site that denitrification is measured.

### **Impact of Fresh Water Diversion on Reducing Nitrate Levels in Mississippi River Waters**

We will, on a monthly basis, monitor change in nitrate levels in Mississippi River from point of diversion with distance into Barataria Basin and Breton Sound estuaries channels. The specific number of sampling points will be determined based on a preliminary survey establishing nutrient gradients. Based on the established nitrate gradient, with distance into the estuary, we will select 3 to 4 sites for measuring denitrification rates using the technique described earlier. In addition nitrous oxide emission to the atmosphere will be measured seasonally along the gradient.

The vertical  $N_2O$  flux density will be estimated by monitoring accumulation of the gas beneath chambers placed over the sediment or water surface (Smith et al. 1983). Triplicate determinations will be made at each site on each sampling date. The chambers will be fabricated from aluminum cylinders, 41 cm internal diameter and have an effective internal height of 10 cm above the water or sediment surface.

The chamber will be supported by styrofoam collars positioned such that 5 cm extends below the air/water interface. The chambers will be shaded at all times thereby minimizing the air temperature increase within the chamber during the sampling period. The chamber used on the open water bodies will be insulated with liquid foam and covered with a reflective space blanket. The tops of the chambers will be fitted with an air sampling port, constructed from a 0.6 cm Swagelok bulk-head union modified for gas chromatographic septum penetration and a vent port constructed from a 1.2 cm Swagelok bulk-head union. An open ended tube (0.6 cm O.D. x 100 cm L) will be attached to the vent port, thereby allowing pressure equilibration during sampling. Aliquots ( $10\text{ cm}^3$ ) of the internal atmosphere will be withdrawn at 15 min. intervals (0-120 min) into glass syringes sealed with 3 way plastic valves (Dowdell et al. 1972). The samples will be collected by passing an 8 cm hypodermic needle through the air sampling port.

The  $N_2O$  content of the gas samples will be measured by gas chromatography techniques (Smith et al. 1981). Nitrous oxide calibration standards will be analyzed repeatedly through all analysis.  $N_2O$  fluxes will be computed from the concentration increase within the internal atmosphere of the chamber (Smith et al. 1982).

### **Products**

The research proposed will provide an understanding of the significance of denitrification in removing elevated nitrate concentrations entering coastal wetlands as a result of Mississippi River freshwater diversion. We acknowledge that this research will not address all issues necessary for reducing nitrate levels and improving water quality in Louisiana offshore areas experiencing hypoxia. However, data generated from the proposed studies will contribute in part to addressing water quality issues and reducing



nutrient levels in the lower Mississippi River drainage basin. Measured denitrification rates will be extrapolated to equivalent surface area for estimating regional denitrification losses and compared to nitrate loading. The research will quantify processing capacity for nitrate in Mississippi River water being diverted into Louisiana coastal wetlands designed to combat land loss. A comprehensive final report will be provided upon completing all research efforts. Results will also be disseminated through publishing findings in referred journals.

Experiment	WORK SCHEDULE							
	2000				2001			
	1	2	3	4	1	2	3	4
Establish Field Transect		X						
Collect Sediment Samples for Calibration of Denitrification Techniques				X	X			
Acetylene Inhibition Assays					X	X		
Direct Measurements of Denitrification (N-15 Labelling)				X	X	X		
Isotopic Dilution Estimates of Nitrification-Denitrification					X	X		
Labile Carbon Relationship to Denitrification				X	X	X		
Data Analysis and Synthesis					X	X		
Final Report Preparation								X
<b>Related Research</b>								

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